



Sem-based characterization of *Psylliodes valida* weise, 1889 (Galerucinae: Alticini): aedeagus and spermatheca in high detail

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Received: 14 June 2025 / Accepted: 12 November 2025 / Published online: 11 December 2025
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Abstract

The paper presents unknown ultrastructure observed by scanning electron microscope (SEM) of aedeagus, spermatheca and body morphology of *Psylliodes valida* Weise, 1889 (Coleoptera: Chrysomelidae: Galerucinae) from Türkiye. This species, which belongs to one of the genera that is very important in biological control, is a new record for Çorum province and the Central Anatolia Region, where it was collected in August 2023. The genus *Psylliodes* Latreille includes 200 species in the worldwide, while it is represented by 42 species in Türkiye. As known, aedeagus, spermatheca and body morphology are taxonomically important structures. However, before the present study, the structural morphology of these features in *Psylliodes* Latreille had not been addressed in any previous research, leaving a notable gap in the literature. For this reason, ultrastructural and detailed investigations of aedeagus, spermatheca and body morphology of *Psylliodes* Latreille from Türkiye were firstly studied with SEM to contain male and female genital descriptions of *Psylliodes valida* Weise, 1889. Photos in SEM are also given in the text.

Highlights

- Alticini species are significant pests that damage cultivated plants.
- SEM images of *P. valida*'s aedeagus and spermatheca are presented for the first time.
- Studying genital morphology reveals insights into beetle reproductive biology.

Keywords Aedeagus · *Psylliodes valida* · SEM · Spermatheca · Türkiye

Introduction

The fact that almost all species of the Chrysomelidae family feed on plants and some are pests of cultivated plants has made it an important subject of agricultural entomology. Alticini species are among the most important insect pests that cause damage to cultivated plants (Metspalu et al. 2014). The most important economic damage to plants is caused by adults that spend the winter in dormancy underground and feed on aboveground organs, especially leaves, stems, flowers and fruits in the spring (Bodnaryk and Lamb 1991; Tansey

et al. 2009; Metspalu et al. 2014). Adult individuals, which prefer fresh and undamaged leaf surfaces for direct feeding, sometimes come together in hundreds and open many small round or oval shaped holes on the leaves they feed on. In some species, this type of feeding can even result in the complete disintegration of the leaf blade (Booth et al. 1990). Soil flea beetles cause direct as well as indirect damage. Adults of some species in particular cause important diseases of various crop plants by virus transmission (Booth et al. 1990). Many Galerucinae and Alticinae larvae are also root miners. Many chrysomelids, especially in Galerucinae, are pests of agricultural crops, and some Bruchinae damage stored seeds (e.g. beans, peas) (Campbell et al. 1989). In addition to their agricultural and economic importance, Alticini represent an important taxonomic group that should be studied primarily due to their high population density. In addition, some studies conducted in Europe and Western America on the biological control of *Lepidium draba* have reported that one of the most effective natural enemies used is *Psylliodes wrasei*,

Handling Editor: Martina Sombetzki

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belonging to the Chrysomelidae family (Cripps et al. 2006; Hinz et al. 2008; Talmaciu et al. 2010). The first record of *P. wrasei* as a potential natural enemy of *L. draba* in Turkey was made by Leonardi and Arnold (1995). The main host of the insect was stated as *L. draba*, a problematic weed in various habitats and many agricultural ecosystems. Later, in a study conducted on the northern shores of Lake Bafa by Aslan et al. (2014), it was reported that *P. wrasei* fed on *L. draba* as a host plant and caused serious damage.

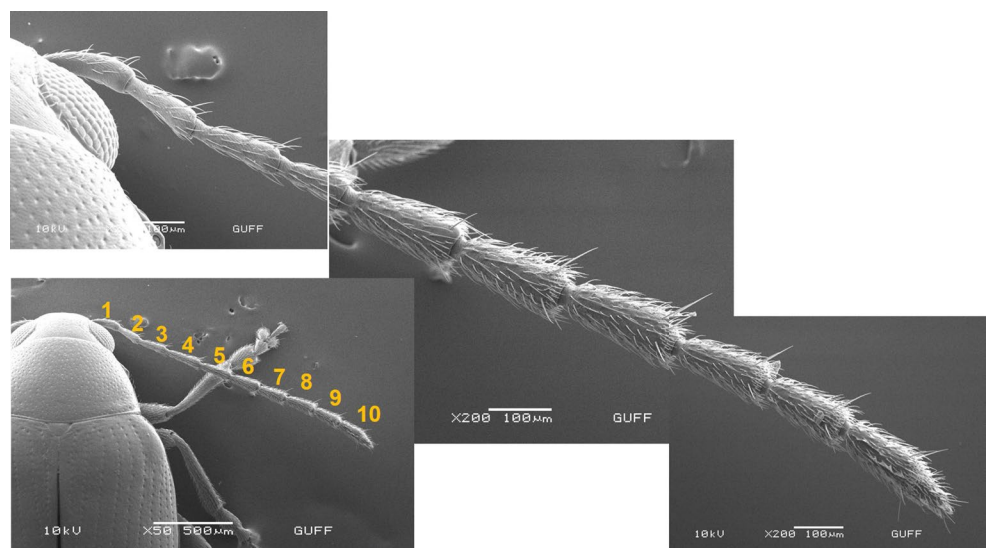
The genus *Psylliodes* Latreille has approximately 200 species in five subgenera worldwide (Konstantinov and Vandenberg 1996; Bezdek & Sekerka, 2024). In Türkiye the genus is represented with 53 species of 3 subgenera. These species are: *Minicnema* Nadein 2007 belonging to the subgenus *Psylliodes elliptica* Allard, 1862; *Psylliodes* Latreille, 1829 belonging to the subgenus 51 species as *Psylliodes aerea* Foudras, 1860, *Psylliodes affinis* Paykull, 1799, *Psylliodes anatolica* Gök & Çilbıroğlu 2004, *Psylliodes arista* Iablokoff-Khnzorian, 1962, *Psylliodes attenuata* Koch, 1803, *Psylliodes ausoniae* Leonardi, 2013, *Psylliodes brisouti* Bedel, 1898, *Psylliodes cerenae* Gök, Doguet & Çilbıroğlu, 2003, *Psylliodes chalcomera* Illiger, 1807, *Psylliodes chrysocephala chrysocephala* Linnaeus, 1758, *Psylliodes circumdata* W. Redtenbacher, 1842, *Psylliodes cuprea* Koch, 1803, *Psylliodes diversicolor* Nadein, 2006, *Psylliodes drusei* Furth, 1984, *Psylliodes dulcamarae* Koch, 1803, *Psylliodes festae* Doderö, 1925, *Psylliodes gibbosa* Allard, 1861, *Psylliodes hospes* Wollaston, 1854, *Psylliodes hyoscyami* Linnaeus, 1758, *Psylliodes illyrica* Leonardi & Gruev, 1993, *Psylliodes inflata* Reiche & Saulcy, 1858, *Psylliodes instabilis* Foudras, 1860, *Psylliodes isatidis* Heikertinger, 1912, *Psylliodes kiesenwetteri* Kutschera, 1864, *Psylliodes laticollis* Kutschera, 1864, *Psylliodes littoralis* Biondi, 1997, *Psylliodes longicollis* Weise, 1900, *Psylliodes luteola* O. F. Müller, 1776, *Psylliodes magnifica*

Gruev, 1975, *Psylliodes marcida* Illiger, 1807, *Psylliodes milleri milleri* Kutschera, 1864, *Psylliodes napi* Fabricius, 1792, *Psylliodes ozisiki* Leonardi and Arnold 1995, *Psylliodes pallidicolor* Pic, 1903, *Psylliodes pallidicornis* Heikertinger, 1921, *Psylliodes persica* Allard, 1867, *Psylliodes picina* Marsham, 1802, *Psylliodes pyritosa* Kutschera, 1864, *Psylliodes ridenda* Nadein, 2008, *Psylliodes saulcyi* Allard, 1867, *Psylliodes taurica* Leonardi, 1971, *Psylliodes testaceoconcolor* Heikertinger, 1926, *Psylliodes thlaspis* Foudras, 1860, *Psylliodes toelgi* Heikertinger, 1914, *Psylliodes tricolor* Weise, 1888, *Psylliodes valida* Weise, 1889, *Psylliodes vindobonensis* Heikertinger, 1914, *Psylliodes wachsmanni* Csiki, 1903, *Psylliodes wrasei* Leonardi and Arnold 1995, *Psylliodes yalvacensis* Gök, 2005; one species as *Psylliodes reitteri reitteri* Weise, 1888 in *Semicnema* Weise, 1888 (Bezdek & Sekerka 2024).

The most important feature that distinguishes the *Psylliodes* genus from other genera is that the number of antennomeres are 10 (Fig. 1). It is easily distinguished from other genera with this feature (Warchalowski, 2010). The subgenus (*Psylliodes* Latreille, 1829) in which the species is located have the following characteristics that differentiate it from the other two subgenus; Mandible wide, weakly curved, not sickle; hind tibia not saw-shaped; hind tarsus attached near apex of tibiae (in apical 1/3 or less), body with more or less rounded margins.

Psylliodes valida Weise, 1889 is of SW Asiatic (Anatolo-Caucasian) origin and recorded only from Erzurum in Eastern Anatolian Region of Türkiye and is the 2nd record from Çorum province with this study. Çorum province is located at the intersection of the Central Black Sea Region and the Central Anatolia Region, in the east extension of the Köroğlu Mountains and the Central Kızılırmak section of Central Anatolia. Therefore, this species is a new record for the Central Anatolia Region.

Fig. 1 Antenna structure of *Psylliodes valida* Weise, 1889



Psylliodes valida Weise, 1889 The vertex and pronotum are more coarse and densely punctuated (Fig. 2), the pronotum is wider and convex in the anterior part; the body is black, with a greenish sheen. Tegmen modified; spermatheca with long collum and nodulus; ductus weakly curved, forming no loops (Nadein 2007). In 2007, Nadein made a study showing key morphological differences among species of the genus *Psylliodes* from a wide area, including drawings of aedeagus and spermatheca and a few photographs. In this study, some remarkable parts of the male (aedeagus) and female (spermatheca) genitalia and body morphology of the species *Psylliodes valida* Weise, 1889 will be described for the first time from Türkiye using SEM microscopy.

However, despite the agricultural, ecological, and biological control relevance of *Psylliodes*, the broader taxonomic and evolutionary significance of morphological characters especially genital structures has received insufficient attention in the literature. Detailed examination of these structures is essential for distinguishing closely related species, clarifying species boundaries, understanding phylogenetic relationships, and improving the systematic framework of the genus.

Materials and methods

The available specimens a total of 40 specimens for the present work were collected in Çorum provinces from Türkiye in August 2023. The specimens were deposited at Gazi

University (Turkey, Ankara). Species were collected with atrap, aspiratör and hand on the host plant.

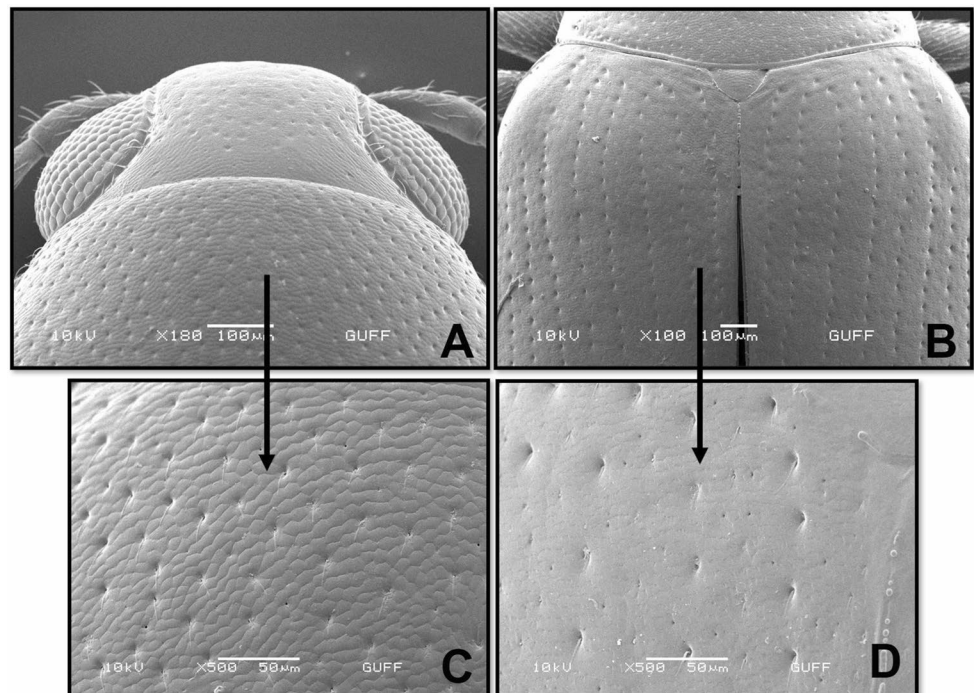
The aedeagus and spermatheca were extracted from the abdomen, and the remaining tissue was carefully removed using fine tweezers. For microscopic analysis after cleaning, the samples were immersed in 70% ethanol and examined with an Olympus SZX7 stereomicroscope and a Leica Z-16 APO stereomicroscope. For scanning electron microscopy (SEM), the cleaned samples were dehydrated with an ascending series of ethanol (70%, 80%, 90%, and 100%) followed by air drying. Subsequently, the specimens were mounted onto SEM stubs using double-sided adhesive tape, coated with gold using a Polaron SC 502 Sputter Coater, and analyzed with a JEOL JSM 6060 SEM at 10 kV at the Prof. Dr. Zekiye Suludere Electron Microscopy Center at Gazi University.

Results and discussion

Psylliodes valida Weise, 1889

Material examined: Çorum prov.: Bayat, exit of Eskialibey village, 40°31'N 34°09'E, 08.VIII.2023, 10 specimens; Bayat, exit of Eskialibey village, 40°31'N 34°09'E, 09.VIII.2023, 5 specimens; Bayat, between Eskialibey-Bayat, Karapınar, 40°30'N 34°08'E, 10.VIII.2023, 10 specimens; Bayat, Karapınar, 40°30'N 34°08'E, 10.VIII.2023, 15 specimens.

Fig. 2 A, C. Punctuation on the pronotum and vertex of *Psylliodes valida* Weise, 1889 in SEM; B, D. Regular large and irregular small punctations on the elytra of *Psylliodes valida* Weise, 1889 in SEM

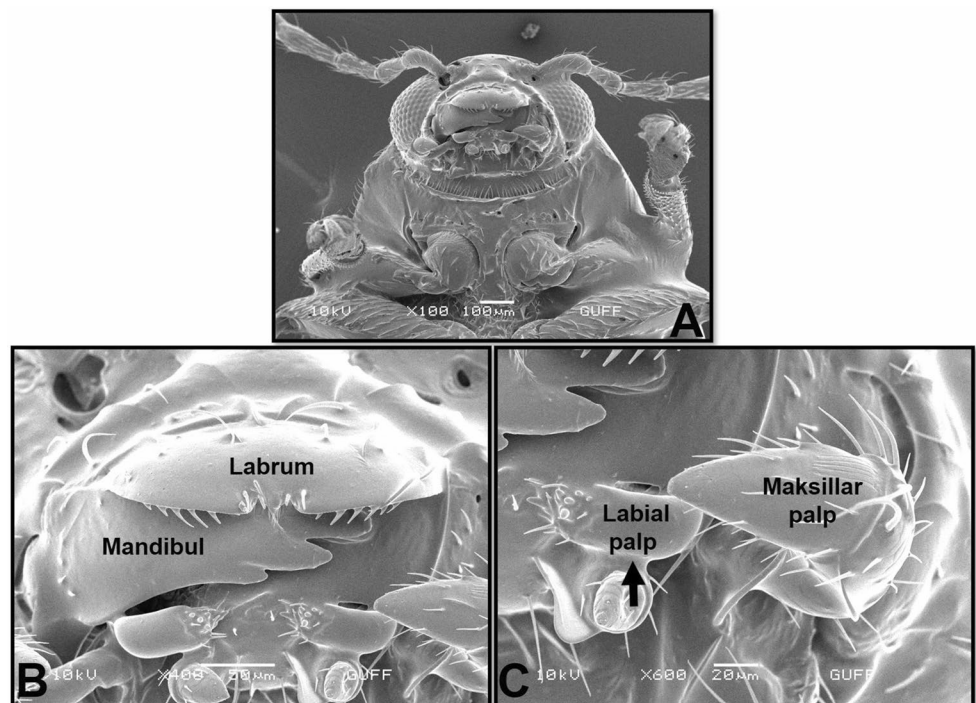


The species is distributed in Europe (Russia: South European Territory) and Asia (Georgia, Türkiye). It has been recorded from one province in Türkiye until now as Erzurum province in Asian Türkiye (Bezdek & Sekerka 2024). In addition, with the present study, it is recorded from Çorum province for the first time. Aedeagus, spermatheca and general morphology of *Psylliodes valida* Weise, 1889 were studied with SEM. Obtaining observations on ultrastructural and detailed morphologies of them are presented as follows.

Some important features that attract attention in the general morphology of the species are;

When we look at the mouth structure, the labrum is quite wide and has two shallow depressions in the middle in the shape of the letter ‘M’. The mandible is 3-lobed at the tip (Figs. 3A-B). The apex of the maxillary palp and labial palp are radially symmetrical, egg-shaped, thin and tapering towards the apex (Fig. 3C). The body and pronotum are generally rounded in shape (Figs. 4A-B). The punctuations on the pronotum and vertex are quite dense and irregular (Figs. 2A and C and 5A-B). When the fine details on the elytra are examined, there are roughly regular rows of punctuations, while there are irregular smaller punctuations in between (Fig. 2B and D). In addition, the hairs on the inner surface of the elytra are short, longer from the inner surface to the outer surface, and the longest hairs are located on the outer edges of the elytra (Fig. 6).

Fig. 3 (A) General view of mouth of *Psylliodes valida* Weise, 1889 (B) Labrum and mandible of *Psylliodes valida* Weise, 1889 in SEM; (C) Maksillar palp and Labial palp of *Psylliodes valida* Weise, 1889 in SEM



Aedeagus: in SEM (Scanning electron Microscope) (Figs. 7A and 8A-B)

A parallel narrowing has occurred at the wide end 1/3 of the median lobe base. The apex of the median lobe is rounded and tongue-like. Median lobe is scattered especially in the anterior half, irregular and sparsely sensillae. There are quite dense sensilla in the terminal region. This situation is more evident in the dorsal part. In addition, there are no sensilla in the dorsal plate (Fig. 7). The endophallus structure and the sensors on it are quite striking structures in SEM images (Figs. 8A).

Spermatheca: in SEM (Scanning electron microscope)

The general shape of vasculum of the spermatheca is more or less cylindrical (Fig. 9A). Spermatheca with long collum and nodulus; ductus weakly curved, forming no loops. Apex of spermathecal pump more or less cylindrical or rounded. Spermathecal pump attached to middle of spermathecal receptacle top. The width of the spermathecal receptacle is the same from base to apex. The spermathecal duct is shortened, shorter than the receptacle. However, the apex of the spermathecal duct is about 1/2 of the chamber and the spermathecal gland is about in the middle of the chamber (Fig. 9B and C). The spermathecal duct connects to the ventrolateral part of the base of the spermathecal receptacle.

Fig. 4 (A) Dorsal view of *Psylliodes valida* Weise, 1889 in SEM; (B) Ventral view of *Psylliodes valida* Weise, 1889 in SEM

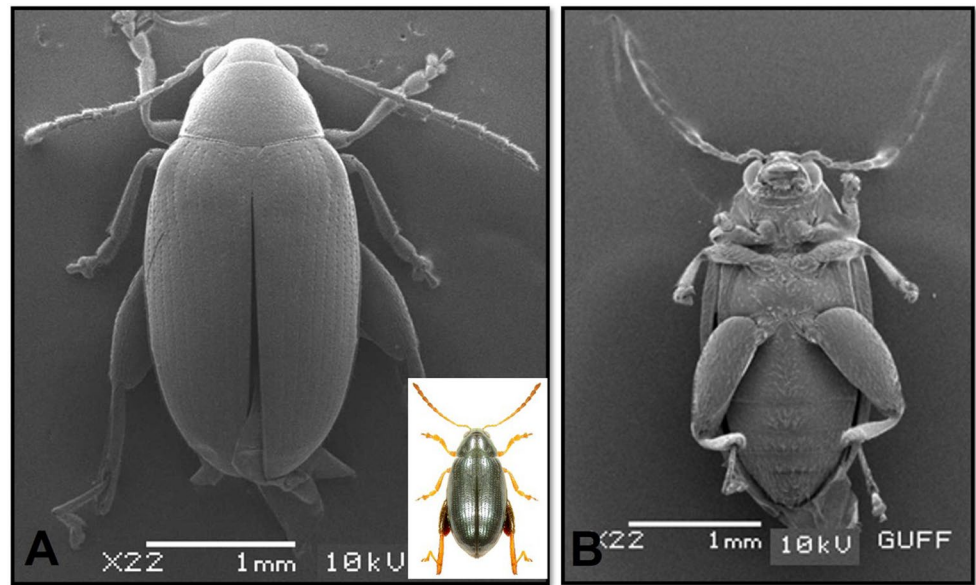
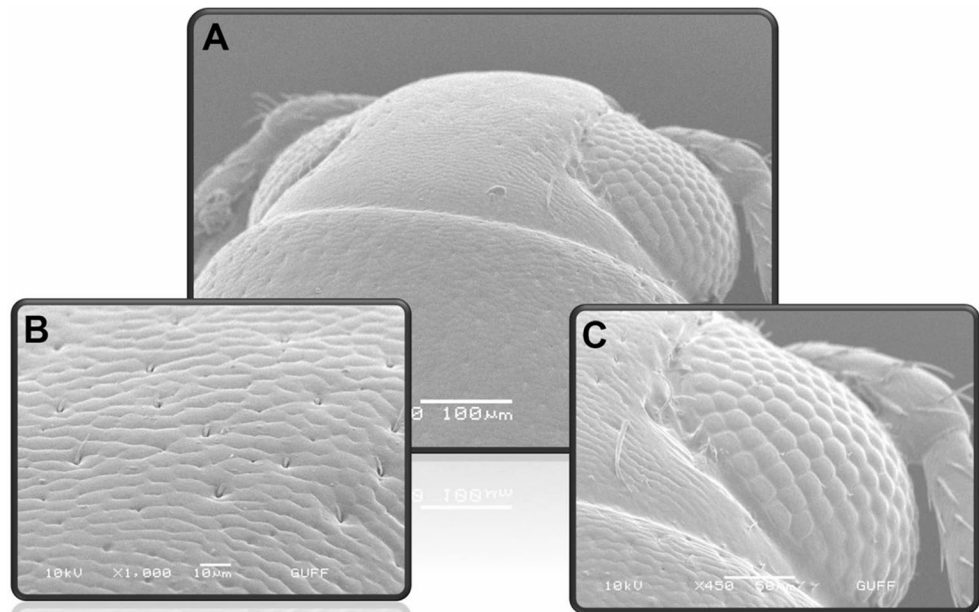


Fig. 5 (A) General view of the vertex of the head of *Psylliodes valida*; (B) Punctuation and surface structure on the vertex of the head. (C) Sensor structures located at the edges of the eye



In Coleoptera, De Marzo (2008) described 5 different spermatheca morphologies, taking into account variations in the presence or absence of spermathecal capsules, ducts, and spermathecal glands. In the most commonly used model, there is a single spermathecal capsule, which is connected to the bursa copulatrix by a spermathecal duct and spermathecal gland. (Suzuki 1988; Snodgrass 1935; Rodríguez-Mirón & Zaragoza-Caballero 2017; Matsumura and Suzuki 2008). In Chrysomeloidea, the spermatheca has been useful for identifying subfamilies, genera and sometimes species (Hernández 1993; Rodríguez-Mirón & Zaragoza-Caballero 2017, Reid 1989; Biondi 2001; Borowiec & Swietojanska 2001; Borowiec and Skuza 2004; Borowiec and Opalinska

2007; Borowiec and Pomorska 2009). According to Rodríguez-Mirón et al. (2017), the spermatheca structure provides distinctive features in the definition of supra-species categories such as genus and subgenus and is of very important taxonomic value for diagnosis.

Conclusion

In Türkiye, the genus *Psylliodes* Latreille is represented by 42 species belonging to 2 subgenera. *Psylliodes valida* Weise, 1889 is of SW Asiatic (Anatolo-Caucasian) origin and recorded only from Erzurum in Eastern Anatolian of

Fig. 6 Hairs on the inner and outer edges of the elytra of *Psylliodes valida* Weise, 1889 in SEM

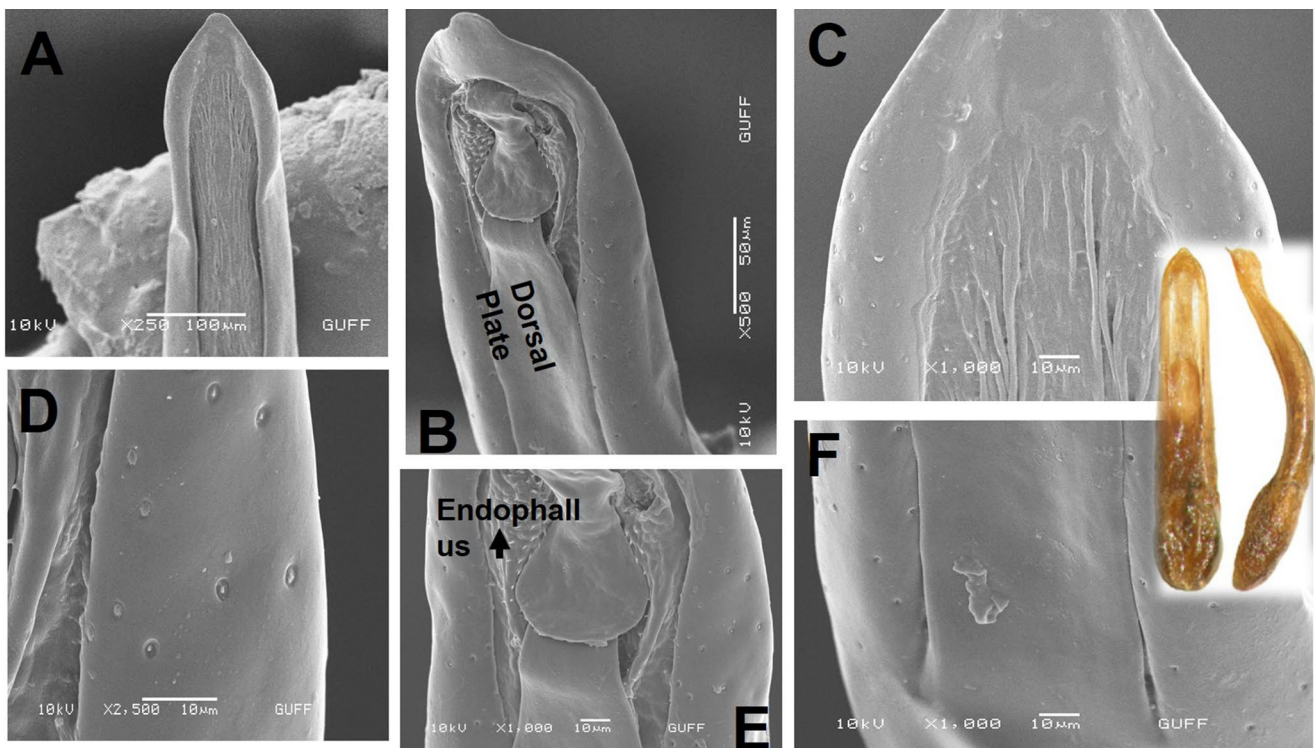
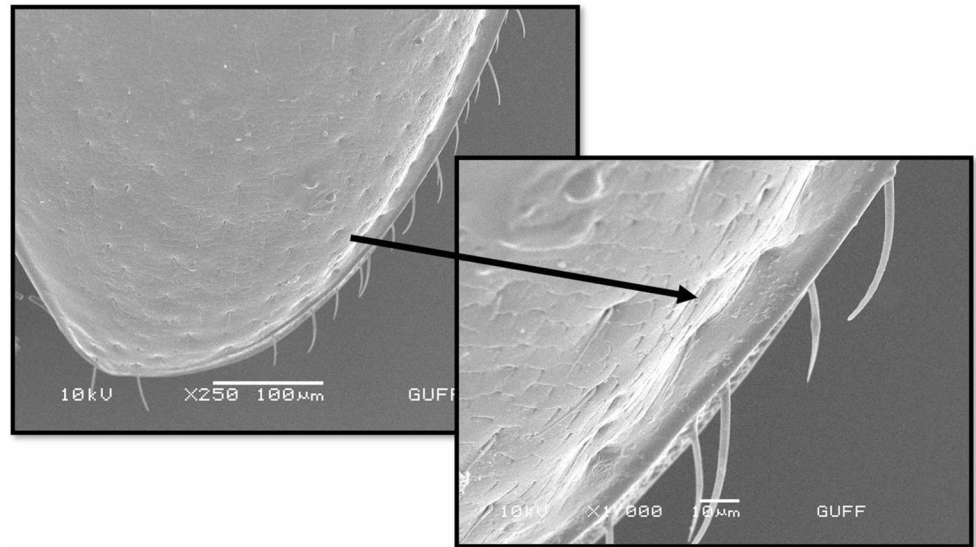


Fig. 7 *Psylliodes valida* Weise, 1889; (A) General shape of aedeagus in dorsal view (scanning electron microscope, SEM); (B) Dorsal Plate of aedeagus in SEM; C, D. Sensillar structure at the end-lateral part

of the median lobe in SEM; E. Endophallus structure that protrudes during mating; F. Sensillar structures seen in the area close to the base of the median lobe

Türkiye and is the 2nd record from Çorum province with this study. Çorum province is located at the intersection of the Central Black Sea Region and the Central Anatolia Region, in the east extension of the Koroğlu Mountains and the Central Kızılırmak section of Central Anatolia. Therefore, this species is a new record for the Black Sea Region of Türkiye. The identification of *P. validus*, which is found

only in Georgia in the Asian continent outside of Türkiye, will provide an opportunity for comparison with other *Psylliodes* species for scientists who are not familiar with this species. However, there is no guiding study especially on their reproductive structure. SEM images of the aedeagus and spermatheca of *P. valida* are presented to the scientific world for the first time in this study.

Fig. 8 A-B. Fine detail of the endophallus structure and the sensor structure

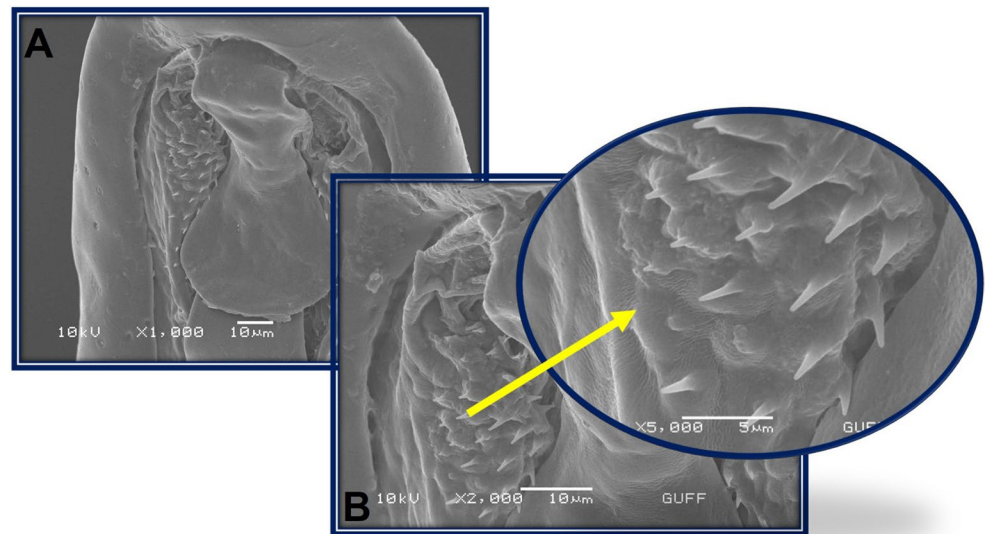
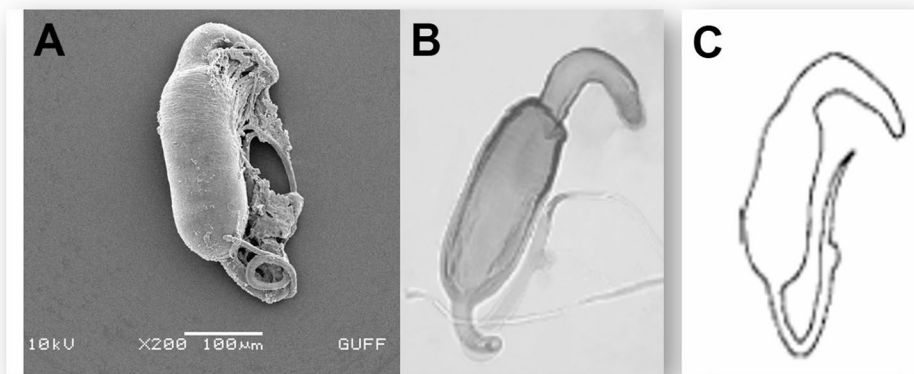


Fig. 9 (A) General view of spermatheca of *Psylliodes valida* Weise, 1889 in SEM; (B) General view of spermatheca of *Psylliodes valida* Weise, 1889 in stereo stereo microscope (only Figure by Nadein 2007), (C) Drawing representation of the spermatheca structure (By Warchałowski 2010)



These structures have specific functions related to sperm storage and fertilization, with variations observed among different species. The differences in spermathecal morphology may be linked to ecological niches, mating behaviors, and the evolutionary pressures faced by various Coleoptera groups. By studying these morphological variations, we gain insights not only into the reproductive biology of beetles but also into their evolutionary history and adaptations.

Acknowledgements I thank Assoc. Prof. Dr. Damla Amutkan MUTLU (Gazi University Faculty of Science, Developmental Biology Department) for her tremendous support in the process of photographing the study material with a scanning electron microscope.

Funding “The author received no financial support for the research, authorship, and/or publication of this article.”

Declarations

Competing interest The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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